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Comparative efficacy of serological tests for diagnosis of ruminant brucellosis

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Abstract
Brucellosis is an alarming zoonosis prevalent globally. This study detected *Brucella* antibodies in ruminants' serum samples and compared the efficacies of different serological tests like Rose Bengal Plate Test (RBPT), Serum Tube Agglutination Test (STAT) and Indirect Enzyme-linked Immune Sorbent Assay (i-ELISA). Of the 463 total serum samples tested, 16 (3.46%), 48 (10.37%) and 28 (6.05%) samples were found positive with RBPT, STAT and i-ELISA, respectively. Diagnostic sensitivities, specificities, positive and negative predictive values, False Discovery Rate (FDR), False Omission Rate (FOR), likelihood ratios, odds ratio and concordance of RBPT and STAT were calculated. Agreement between various serological tests was calculated using kappa statistics. Substantial agreement was obtained between i-ELISA and RBPT (κ=0.67) whereas fair agreement was evident between i-ELISA and STAT (κ=0.26). Seropositivity was confirmed by i-ELISA whereas highest percentage was obtained by STAT. Organised sector scored higher compared to unorganised sector. The results of the study indicate that STAT can be used for screening purpose but because of its false negative results it is not reliable. On the other hand, in absence of isolation and identification of brucellae, i-ELISA can be used as a standard reference test because of its higher sensitiveness and specificity.

Keywords: Brucellosis, i-ELISA, sensitivity, specificity, kappa value

Introduction
Brucellosis is one of the most important contagious and communicable bacterial diseases, with a worldwide distribution and is endemic in most part of world especially the developing countries. It is considered a remerging though neglected zoonosis with high rates of morbidity and lifetime sterility. The incidence of brucellosis cases is increasing over the recent years especially in developing countries due to poor management, limited resources [9] and increased trade and frequent movement of livestock [19]. The disease is equally important with respect to veterinary and public health aspects and causes significant morbidity and enormous economic losses [12, 24]. *Brucella abortus* and *Brucella melitensis* are considered to be the most virulent brucellae of large and small ruminants, respectively. The disease in animals mostly characterised by abortion storms in the herd, infertility and low milk yield. In humans, it is manifested as an acute, subacute or chronic febrile illness causing night sweats, arthritis and occasional abortions in females [11]. The disease is difficult to control because of its insidious nature and chronic clinical manifestations. Therefore, a battery of serological tests is being preferred viz., Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Enzyme Linked Immunosorbant Assay (ELISA) etc. for the detection of brucellosis. The present study is based on different serological tests for assessing the seroprevalence of brucellosis in ruminants of Brij region in Uttar Pradesh.

Materials and Methods
Serum samples: Among the total 463 serum samples, cattle (90), buffalo (125), sheep (135) and goat (113) samples were taken. The samples were collected from private farms, veterinary hospitals, villages and ILFC of DUVASU, Mathura. About 5 ml of blood was collected aseptically from the jugular vein of individual animal in serum activator vial and transported to the laboratory on ice. The serum further was collected by centrifugation at 3,000 rpm for 5 minutes and stored at –20 °C till performance of laboratory tests.

Serological Tests

Rose Bengal Plate Test: The Rose Bengal Plate Test antigen used for the test was obtained from Indian Veterinary Research Institute, Izatnagar, UP and the test was followed according to the procedure described by Alton *et al.* [1] A positive reaction shows clumping or agglutination whereas negative reaction shows no clumping or agglutination.

Standard Tube Agglutination Test: The test procedure was performed accordingly as instructed in the protocol given by Alton *et al.* [1] The antigen used was procured from BP Division, IVRI, Izatnagar. A titre of ≥80 IU was taken as positive in large ruminants while ≥40 IU in case of small ruminants.

Indirect Enzyme Linked Immunosorbent Assay: The test was performed using IgG based kits for large and small ruminants separately which were procured from ICAR National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bangalore, Karnataka. The test procedures were followed according to the manufacturer's protocol. The optical density (OD) for both the cases was measured at 492 nm. Thereafter the per cent positivity (PP) value of test samples was calculated individually using the formulae below.

$$PP = \frac{Average\ OD\ value\ of\ test\ serum}{Median\ OD\ value\ of\ positive\ control} \times 100$$

On calculation, PP values >54% were considered as positive and <54% as negative for brucellosis according to the kit guidelines.

Results and Discussion

On analysis of different serum samples from different species of ruminants for brucellosis, this cross-sectional study came up with caprine being the highest *Brucella* positive species. It revealed 10.62% positivity by i-ELISA (Table 1).

Table 1: Seropositivity of Brucellosis among Different Species of Animals

Species	Positive Number	% positivity	χ^2 test
Cattle	9	10	NS
Buffalo	5	4	NS
Sheep	2	1.48	6.206*
Goat	12	10.62	13.27*

χ^2 stands for chi-square; * significantly differ within the three serological tests employed in specific species (P<0.05); NS stands for not significant.

The present study revealed overall 3.46% positivity by RBPT which was almost similar with Samaha *et al.* [8] who detected 3.5% of positivity. Kumar *et al.* [18] recorded 5.65%, Sharma *et al.* [22] reported 7.73% and Din *et al.* [5] recorded 13.33% seropositivity by RBPT. A much higher seroprevalence of 25.80% was recorded by Kaltungo *et al.* [8] whereas a lower percentage of 1.71% had been reported by Sharma *et al.* [23] The results of the RBPT was taken within the prescribed duration in order to escape false positive results which occurs principally because of fibrin clotting.

An overall outcome of 10.37% was revealed by STAT in this investigation. Similar findings of 10% was revealed by Padher *et al.* [14] Likewise, comparatively a higher seroprevalence of brucellosis by STAT was shown by Bertu *et al.* [4] Salama *et al.* [20] and Awandkar *et al.* [3] as 16.02%, 27.00% and 23.8% respectively. In contrast lower positivity from the current study of 9.33% was reported by Din *et al.* [5] 3.42% by Sharma *et al.* [23] and 5.43% by Kumar *et al.* [10

6.05% overall seropositivity was detected by i-ELISA test in this study. Similar 5.71% seroprevalence was found by Sharma *et al.* [23] using i-ELISA. Kumar *et al.* [10] revealed 5.98% positivity, Rahman *et al.* [16] detected 1.25% and Tayshete, [25] found 4.00% seroprevalence which were lower than this finding. On the other hand, works of Reddy *et al.* [18] and Valarmathy *et al.* [26] were having higher scores as 9.52% and 30.04% respectively.

Besides, in this cross-sectional study, comparative efficacy of serological tests employed (RBPT and STAT) for the detection of ruminant brucellosis was also examined. The sensitivity, specificity, positive predictive value, negative predictive value, false discovery rate (FDR), false omission rate (FOR), positive and negative likelihood ratios, odds ratio, concordance and agreement between the serological tests (to evaluate the kappa value) were calculated taking i-ELISA as the standard test. i-ELISA was considered as gold-standard test due to its specific and precise detection of IgG, IgM and IgA antibodies [2]. All these parameters are represented in Table 2.

Table 2: Relative Efficacy of Serological Tests Employed

Test	RBPT	STAT
Sensitivity	53.57%	42.86%
Specificity	99.77%	91.72%
Positive PV	93.75%	25%
Negative PV	97.09%	96.14%
FDR	6.25%	75%
FOR	2.91%	3.86%
LR Positive	232.9	5.18
LR Negative	0.47	0.62
Odds Ratio	500.77	8.31
Kappa Value	0.67	0.26
Status of Kappa Agreement	Substantial	Fair
Concordance	96.98%	88.77%

Further on analysis of the total data according to their rearing practice and herd size, viz., organised and unorganised sectors, it was detected that organised sector showed 75% positivity compared to unorganised sector expressing 25% positivity by i-ELISA. Hence, an overall significant variation (P<0.05) was recorded in this aspect. Moreover, the studies of Nasir *et al.* [13] Kachhawaha *et al.* [7] Ramesh *et al.* [17] and Sharma *et al.* [22] resemble with the present study. The occurrence in organized rearing system may be because of easily transmission of infectious agent between susceptible and infected animals due to high population density. Besides, poor managemental practices can also maximise the effect of brucellosis in organised farms.

Conclusion

On the basis of this study the status of brucellosis in Brij region is annoying and its prevalence is quite significant. Side-by-side organised sector possesses the more danger of the disease. Moreover, economic losses due to brucellosis are extensive worldwide. Therefore, a couple of tests should be considered for the confirmed detection of brucellosis. Though i-ELISA is superior serological test in brucellosis diagnosis but its cost effectiveness and sophistications may become the challenges of its way to routine use.

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